

PP274—MEMANTINE PREVENTS THE DEVELOPMENT OF NEUROPATHIC PAIN WHEN GIVEN BEFORE SURGERY

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Introduction: N-methyl-D-aspartate receptor (NMDAR) antagonists are used for postsurgery neuropathic pain states, but severe side effects limit their clinical use. Memantine, when given after surgery, shows contradictory results as regards neuropathic pain alleviation. **Patients (or Materials) and Methods:** Memantine is administered in animals before or after spinal nerve ligation (SNL) to evaluate the antinociceptive/cognitive effects and associated molecular events (including phosphorylated-Tyr1472NR2B subunit). SNL animals received memantine or saline 4 days before (pre-emptive) or after surgery (postoperative) for 7 days, were tested for tactile allodynia, mechanical hyperalgesia, cognitive function and spinal cord molecular events, and results were compared (significance $P < 0.05$).

Results: Postoperative memantine had no beneficial effect on tactile allodynia, mechanical hyperalgesia, or spatial memory, and molecular expression of NR2B subunit was significantly increased. Pre-emptive memantine prevented the development of mechanical hyperalgesia, tactile allodynia, and impairment of spatial memory; spinal pTyr1472NR2B was not increased.

Conclusion: Blockade of NMDAR by memantine 4 days before surgery rather than postoperatively is a promising strategy in animals to alleviate neuropathic pain development and impairment of cognitive function. The pivotal role of pTyr1472NR2B must be studied further, and confirmation of these findings in patients would constitute a giant footstep in the prevention of neuropathic pain that frequently follows surgical procedures.

Disclosure of Interest: None declared.

PP275—CHITOSAN/POLYMER NANOPARTICULATE CONTROLLED RELEASE SYSTEM FOR IBUPROFEN

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Introduction: With >25% of the American population experiencing daily pain and the number of new drugs still in the single-digit area, it is becoming more and more of an important health problem to come up with new or improved analgesis compounds. Our paper proposes a new approach for obtaining an efficient controlled-release system for the pain treatment via innovative polymeric nanoparticles by using an interfacial condensation method between a natural polymer, chitosan (CS) and a synthetic one (poly (maleic anhydride -alt- vinyl acetate), (poly (MAVA)).

Patients (or Materials) and Methods: Chitosan, acetone, Ibuprofen (IBU), hexane, and surfactants were used (Sigma Aldrich). The alternant copolymer – poly (MAVA) was synthesized by radical copolymerization and characterized in our laboratory. The nanocapsules were characterized and investigated by Fourier transform infrared spectroscopy (FTIR), Scanning Electron Microscope (SEM), Transmission

Electron Microscope (TEM), thermogravimetric analysis (TGA), and differential scanning calorimetry (DSC). In vitro IBU loading and release was also investigated. In vivo testing of IBU/nanocapsules was evaluated by hot plate test and tail flick test, behavioral tests that quantify the thermal nociception. Adult male Swiss mice in different groups of 8 mice each were intraperitoneally injected with one of the following formulations: 0.9% saline for a control group; ibuprofen for positive control group (100 mg/kg b.w.); ibuprofen-loaded nanocapsules (MAVA-CS-9) for test group (100 mg/kg b.w. equivalent ibuprofen). Inhibition to pain stimuli was then calculated. **Results:** Zeta potential determinations indicated a good stability of the nanocapsules in aqueous solutions. Scanning electron microscopy confirmed the nanometric dimensions and the spherical shape of the nanocapsules. The thermal properties achieved by DSC and ATG showed the nanocapsules' good thermal stability. In addition, these systems present a good swelling capacity, which is influenced by the reaction parameters taken into account in this study. The drug-loading and -release capacity (studied for ibuprofen sodium salt used as water-soluble drug) is controlled by the diffusion speed through the polymeric membrane and is influenced by the swelling degree. Although ibuprofen alone has a half-life of 1 to 3 hours, and previous studies showed an analgesic improvement of their nanoparticle formulations in the 1- to 2-hour range, our system has a time interval of 2 to 6 hours of analgesic efficacy, an increase of up to 6 times the reference ibuprofen formulation.

Conclusion: We have proved the possibility of a sustained-release system for central pain inhibition (from 2 to 24 hours) versus 0 to 2 hours ibuprofen control. Future variations in the ibuprofen load of the nanocapsules could yield potentially significant commercial applications with improved efficacy and safety profiles.

Disclosure of Interest: None declared.

PP277—INCREASE IN MORPHINE ANALGESIC EFFECT WHEN CO-ADMINISTRATED WITH BILE ACID DERIVATES

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Introduction: It is known that bile acids improve the absorption, bio-availability, and pharmacodynamic characteristics of some drugs. Because morphine analgesia is produced by activation of opioid receptors within the central nervous system at both spinal and supraspinal levels, and because morphine molecule contains 3 polar groups, and as such is hard to transfer through the blood-brain barrier, the aim of the study was to examine the potential influence of bile acids derivatives, namely sodium salt of monoketocholic acid (MKH-Na) and methyl ester of monoketocholic acid (MKH-Me) on analgesic effect of morphine.

Patients (or Materials) and Methods: White male mice of NMRI-Haan strain, with body weight of 20 to 24 g, were used in this study. Analgesic effect of morphine was estimated by the hot plate method. Analgesic effect of morphine (2 mg/kg) (administered by subcutaneous and intramuscular route of administration) with and without pretreatment with MKH-Na (4 mg/kg) and MKH-Me (4 mg/kg) was measured.

Results: Administration of MKH-Me before subcutaneous administration of morphine increased the morphine analgesic effect, but the increase was not statistically significant. At the same time the administration of MKH-Na did not affect morphine analgesic effect. Analgesic effect of morphine was increased when morphine

was administered intramuscularly 20 minutes after MKH-Me administration. When compared with group of animals treated only with morphine, statistically significant increase in analgesic effect was detected 10, 30, 40, and 50 minutes after morphine administration ($P < 0.05$). Pretreatment with MKH-Na did not affect morphine analgesic effect.

Conclusion: According to results of this study, it can be presumed that after intramuscular morphine administration methyl ester of monoketocholeic acid increases morphine transport into the central nervous system and consequently analgesic effect as well. Further research on bile acids-morphine interaction both in vitro and in vivo is necessary to completely elucidate the mechanism of this interaction and increase in morphine analgesic effect.

Disclosure of Interest: None declared.

PP278—BILE ACID INDUCED CHANGES IN THE EXPRESSION OF MIRNAS AND GENES INVOLVED IN DRUG METABOLISM AND DISPOSITION

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Introduction: Bile acids (BAs) are essential for the absorption, transport, and distribution of dietary lipids, vitamins, and xenobiotics. As transcription factor ligands (eg, of FXR), they influence signaling pathways regulating lipid, glucose, and energy homeostasis.¹ First studies indicate that BAs are also able to influence the expression of microRNAs (miRNAs), a class of small noncoding RNAs that is able to inhibit protein translation.^{1,2} Several different hepatic disease states as well as the intake of certain hepatotoxic drugs can induce cholestasis, a condition associated with elevated bile acid levels in hepatocytes.

We aimed to comprehensively investigate the impact of BAs on the human mRNAome and miRNAome, focusing on the question, to which extent elevated BAs may have the potential to influence the expression of genes involved in drug metabolism and disposition and signaling pathways driven by miRNAs.

Patients (or Materials) and Methods: Two batches of primary human hepatocytes were treated with 50- μ M chenodeoxycholic acid (CDCA) or vehicle (DMSO) for 48 hours. Global mRNA and miRNA profiling was performed using next-generation sequencing. Adapter-ligated RNA samples were reverse-transcribed and enriched by PCR. After cluster generation (TruSeq PE Cluster Kit v3-cBot-HS (Illumina)) sequencing was performed on the Illumina HiSeq 2000 (TruSeq SBS Kit v3-HS (Illumina)). RNA-reads were quality-checked (fastqc) and aligned to the genome (tophat v1.3.3). The GLM procedure (edgeR, R package) was used for statistical analysis comparing the mRNA and miRNA expression values across the different conditions.

Results: A total of 702 genes were significantly decreased, 1345 genes and 71 miRNAs were significantly increased in expression in BA-treated cells. Besides classical FXR targets involved in BA transport and metabolism (eg, CYP7A1, BSEP) that served as positive controls for repression and induction by BAs, respectively, many genes coding for proteins involved in drug metabolism appear to be affected and in many cases down regulated by BAs. Examples include genes encoding members of the CYP1A, CYP2C and CYP3A and the SLC transporter family as well as members of the GST, SULT, and UGT family. Notably members of the miRNA families 320 and 548 appeared to be strongly regulated by CDCA.

Conclusion: BAs have the potential to significantly modulate the expression of genes involved in the biotransformation of drugs and, thus, may have a significant impact on drug metabolism and disposition.

The detection of differentially expressed miRNA molecules extends the spectrum of regulatory pathways influenced by BAs.

Disclosure of Interest: None declared.

References

1. Song, et al. *J Lipid Res.* 2010;51:8.
2. Castro, et al. *J Hepatol.* 2013;58:119-125.

PP279—CARDIO-HEPATIC SYNDROME AND THERAPEUTIC EFFICACY OF NAD-CONTAINING DRUG

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Introduction: Liver dysfunction is frequent in CHF and characterized by a predominantly cholestatic enzyme profile that is associated with disease severity and prognosis. Thus, we propose a cardio-hepatic syndrome in CHF. Future studies are needed to clarify the exact mechanisms of organ interaction. The aim of the study was to evaluate the efficacy of various therapy of CHF to cessation the symptoms of "cardio-hepatic" syndromes.

Patients (or Materials) and Methods: A total of 125 patients with IHD and CHF NYHA class II-III were enrolled in this open, controlled trial. The protocol was approved by local ethical committee. All patients after randomization were included into group; control and main. In the control group, patients were treated by standard combination and in main group additionally received badci (NAD-containing drug) in dose of 180 mg once daily for 14 days. Symptoms of CHF, ECG, EchoCG, and markers of hepatic function were clinically compared. Blood chemistry analysis included the measurements of circulating level of total and direct bilirubin, ALT, AST, γ -glutamyltransferase (GGT), and alkaline phosphatase (ALP), NAD, NAD/NADH levels and the content of proinflammatory cytokines (IL-1 and IL 6) were studied. Data were analyzed with SPSS 10.0 statistical package.

Results: In patients with stable CHF without marked systolic dysfunction, changes of liver abnormalities were common by typically by small amplitude, particularly in those patients with an ejection fraction <35%. Middle elevation of alkaline phosphatase and total bilirubine as well as mild decrease of albumine were observed. The redox potential of NAD/NADH in the plasma was reduced by 21% without significantly changes in the total content of pyridine nucleotide. We postulated that hypoxia might play a pathophysiologic role linking CHF to liver injury. The content of IL6 increase up to 112% in patients with average NYHA class 2.3 (0.2) (according to J. Cohn HF score, 1986) in the mechanism of which leads the ability of IL-6 binds to hepatocytes by interacting with an 80-kd membrane glycoprotein (gp80). Including in the therapy NAD-containing drug, nadcin (180 mg once daily IV) significantly improved hepatic function and cholestatic abnormalities, and as very important decrease up to normal level the content of IL6 and IL-1 β in patients with CHF without pronounced left ventricle dysfunction.

Conclusion: Cessation of abnormalities of the hematologic system and decrease in the content of IL-6, as 1 of the key regulators of the initial steps of liver regeneration and IL-6-dependent signaling response mechanism under treatment with exogenous NAD-containing drug (in form of nadcin) restores the cell redox-potential, occurs beneficial effect on the cytokine system and as a result occurs deremodelling of liver functioning system, opens the new era in the CHF therapy and avoid the cardio-hepatic syndrome formation.

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